SOP Name	Preparation of PBMCs
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1. **SCOPE:** This SOP applies to all staff, visitors, researchers and research staffworking in VACCELERATE affiliated labs.

2. **PURPOSE:** To outline the appropriate policies and standards for isolating andstoring PBMCs from whole blood.

3. **POLICY:** VACCELERATE works within the guidelines and regulations of the EU CT Directive 2001/20/EC, GCP Commission Directive 2005/28/EC, ICH/GCP and with all other local and international applicable regulatory requirements.

4. **ROLES AND RESPONSIBILITIES**
    1. The investigators should stipulate the appropriate processing requirements for all prospective studies with VACCELERATE lab staff prior to the study's initiation.
    2. It is the responsibility of the research personnel carrying out this procedure to ensure that all steps are completed both competently and safely.

5. **DEFINITIONS**

PBMC= peripheral blood mononuclear cell
CPS = Cryopreservation solution

6. **RELATED DOCUMENTS**

SPREP002 Collection and handling of bio materials
SPREP003 Core lab sample receipt and storage
SPREP004 Courier packages
SPREP005 Sample handling
SPREP007 Venepuncture

7. **PROCEDURES**

The sample processing requirements for all prospective studies should be discussed with VACCELERATE lab staff prior to the study's initiation. The Pl will select the appropriate PBMC preparation for study requirement.

7.1 STANDARD TECHNIQUE

7.1.1 Recommended Materials:

- Citrate or heparin blood tubes (BD vacutainer 'light blue/dark green'or Sarstedt 'light green')
- Lymphoprep® Alere (ref. 1114547)
- Sterile Phosphate-Buffered Saline (PBS) without calcium or magnesium: Thermo Fisher, 14190-094
- Sterile transfer pipettes
- 10-25ml sterile serological pipette
- Pro-pipette
- CPS (90% HI-FBS – see SOP Reagent preparation, 10% DMSO – Sigma Aldrich, D8418)
- Sterile 15ml and 50ml centrifuge tubes.
- Sterile cryovials
- Mr. Frosty® (Optional)

7.1.2 Procedure:

This procedure is carried out under sterile conditions using a laminar flow hood (biosafety cabinet). Decontaminate the laminar hood by using 70% ethanol to clean the interior surface area. Sterilise any consumables with ethanol prior to placing them in the laminar hood.

1. Dilute whole blood 1:1 with sterile PBS or 0.9% NaCl. Take note of final blood volume.
2. Transfer lymphoprep solution into a sterile 50ml tube at a 1:2 volume of lymphoprep to whole blood. Typically,

10ml Lymphoprep to 20ml diluted whole blood.

3. Using either a sterile transfer pipette or serological pipette, carefully layer the whole blood on to the Lymphoprep solution, (avoid mixing of both solutions).
4. Centrifuge the layered sample preparation at 2000rpm for 25 minutes at RT (18-25°C) with the brake off.
5. Remove the PBMC layer using a sterile transfer pipette and transfer to a 15ml centrifuge tube. Avoid aspirating up any red blood cells (RBCs).
6. Fill tube to 10ml with sterile PBS, vortex and centrifuge for 10 minutes at 1500rpm with slow brake.
7. Discard the supernatant and re-suspend the cell pellet in 1ml of freezing media (90% FBS to 10% DMSO). Transfer 500ul aliquots into pre-labelled cryovials.
8. Place the samples in a -80°C freezer (in Mr. Frosty if available) overnight before transferring to long-term LN_2 storage.

7.2 SepMate technique

7.2.1 Recommended Materials:

- Citrate or heparin blood tubes (BD vacutainer 'light blue/dark green' or Sarstedt 'light green')
- Lymphoprep® Alere (ref. 1114547)
- Sterile PBS
- Sterile transfer pipettes.
- 10-25ml sterile serological pipette
- Pipette driver/gun
- Freezing medium. (90% FBS, 10% DMSO}
- Sterile 15ml and 50ml centrifuge tubes.
- SepMate™ PBMC Isolation tubes
- Sterile cryovials
- Mr. Frosty® (Optional) more info about this product

7.2.2 Procedure:

1. This procedure is carried out under sterile conditions using a laminar flow hood (biosafety cabinet). Decontaminate the laminar hood by using 70% IMS to clean the interior surface area. Sterilise any consumables with IMS prior to placing them in the laminar hood. Sterilise gloved hands using IMS.

2. Pour all whole blood collected for PBMC isolation into one sterile 50ml tube. Pour an equal volume of sterile PBS or 0.9% NaCl solution into the same tube, noting the final volume.

3. Transfer 15mls of Lymphoprep solution into a sterile 50ml SepMate™ tube using a pipette driver and sterile serological pipette. Lymphoprep must be added in through the divider in the SepMate tube slowly and carefully in order to ensure there is no Lymphorep on the outside of the

SepMate divider. Repeat with another SepMate tube.

4. Using a sterile serological pipette, take up half of the PBS/whole blood solution and carefully and gently begin to allow the PBS/whole blood solution to aspirate onto the outer side of the SepMate tube divider. Do this again with the remaining PBS/whole blood solution in the second SepMate tube.

5. Centrifuge the two SepMate tubes at1200g for 10 minutes at RT (18-25°C) with the brake on 4.

6. Following careful removal of the SepMate tubes from the centrifuge, quickly and smoothly decant the top layer into a fresh sterile 50ml tube. Take care not to invert the SepMate tube for more than two seconds. Repeat for the second SepMate tube in another fresh sterile 50ml tube.

7. Add l0mls sterile PBS and 2% FBS solution to each of the tubes, inverting slowly and gently to mix. Centrifuge both tubes for 10 minutes at 400xg with the brake at 4.

8. Discard the supernatant and re-suspend the cell pellet in 10ml PBS and 2% FBS solution by gentle inversion. Centrifuge the tubes again at 400xg for 10 mins with brake at 4.

9. Discard the supernatant and resuspend the pellets in 1ml freezing medium (900ul FBS, l00ul DMSO) and transfer to labelled cryovials. Place the labelled cyrovials in a -80°C freezer (in Mr. Frosty if available) overnight before transferring to long-term LN_2 storage.

8. REVIEW AND REVISION

This SOP should be reviewed at least every two years and revised accordingly.

9. DOCUMENT HISTORY

Version Number	Effective Date:	Summary of changes from previous version:	Edited by: (name and role)